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Target fishing reveals *Pf*PK-1 and *Pf*Rab6 as potential targets of an antiplasmodial 4-anilino-2-trichloromethylquinazoline hit compound

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Abstract

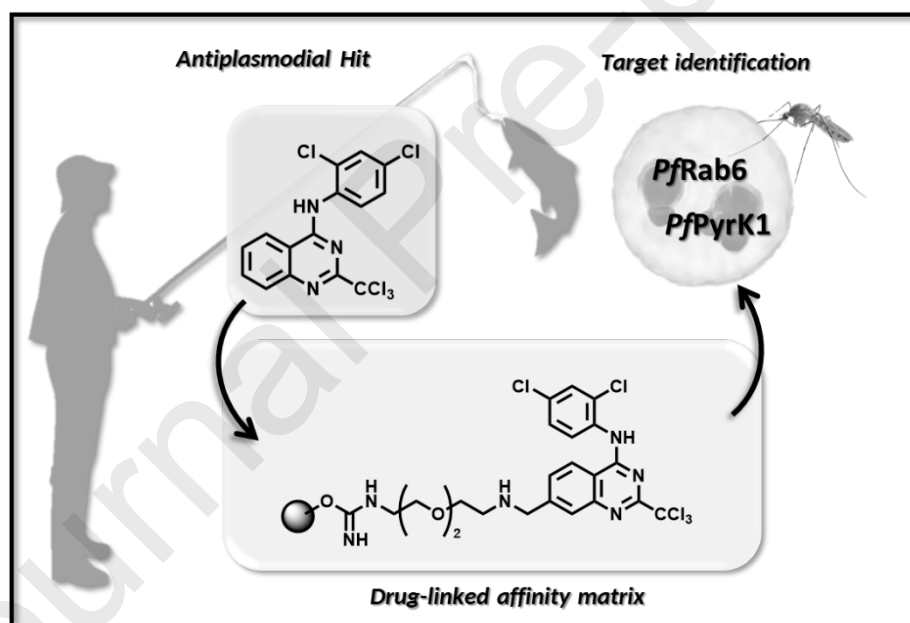
We present investigations about the mechanism of action of a previously reported 4-anilino-2-trichloromethylquinazoline antiplasmodial hit-compound (**Hit A**), which did not share a common mechanism of action with established commercial antimalarials and presented a stage-specific effect on the erythrocytic cycle of *P. falciparum* at $8 < t < 16$ h. The target of **Hit**

A was searched by immobilising the molecule on a solid support *via* a linker and performing affinity chromatography on a plasmodial lysate. Several anchoring positions of the linker (6,7 and 3') and PEG-type linkers were assessed, to obtain a linked-hit molecule displaying *in vitro* antiparasmodial activity similar to that of unmodified **Hit A**. This allowed us to identify the *Pf*PYK-1 kinase and the *Pf*Rab6 GTP-ase as potential targets of **Hit A**.

Keywords

Target fishing, antiparasmodial hit, 2-trichloromethylquinazoline, drug-linked matrix, affinity chromatography, *Pf*PYK-1, *Pf*Rab6

Graphical abstract



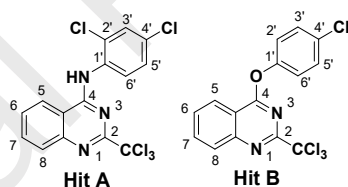
Introduction

With an estimation of 247 million cases in 2021, malaria is the most common parasitic infection worldwide [1]. The sub-Saharan region accounted for 95% of cases, and 4 countries (Nigeria, Democratic Republic of the Congo, Niger, and the United Republic of Tanzania) accounted for 52% of all cases [1]. Worldwide, 619 000 people died from malaria in 2021, among which 76% were children under 5 years. Five species of malaria parasites infect human, *Plasmodium falciparum* being the most lethal and responsible for the cerebral form of the disease. Artemisinin derivatives are the first-line treatment recommended by the WHO against severe forms of *P. falciparum* infections and are used in combination with other antimalarial molecules displaying different mechanisms of action in Artemisinin Combination Therapies (ACTs). Artemisinin resistance manifests clinically as slow parasite clearance; as a result, fewer malaria parasites are killed during the 3 days of the ACTs treatment,

increasing the risk of treatment failure and facilitating selection of partner drug resistance [2]. Artemisinin resistance has been reported for several years in the Greater Mekong Subregion [3], and the recent detection of clinical resistance to artemisinin monotherapy in other regions, including Africa, appears as a major threat to malaria control [4]. There is therefore an urgent need for new antimalarial drugs, with original mechanisms of action and untapped targets to prevent cross-resistance. In this way, MalDA (Malaria Drug Accelerator) is an international consortium whose aim is to improve and accelerate the early antimalarial drug discovery process by identifying new, essential, and druggable targets [5] and Medicines for Malaria Venture (MMV) is currently developing, at a clinical stage, seven new chemical entities displaying original mechanisms of action [6].

Our team has previously reported the antiplasmodial potential of some quinoline [7], quinazoline [8-10] and quinoxaline [11] derivatives bearing a trichloromethyl (CCl_3) group. These include quinazoline **Hits A** and **B** (Table 1), which exhibit mechanism of action different from those reported for 4-aminoquinolines (inhibition of heme crystallization), proguanil (*PfDHFR* inhibition) and artemisinin derivatives (free radical formation followed by heme alkylation). A proven route for target identification involves *in vitro* generation of resistant strains followed by whole-genome sequencing. This strategy has proven to be very effective in identifying the molecular targets of many antimalarial compounds [12-15]. Affinity chromatography using immobilised compounds, combined with mass spectrometry (MS), is another complementary approach to target identification [16-19]. Using this approach, target proteins of several antiplasmodial compounds were already identified [20-22]. Herein, affinity chromatography was used to search for the target of 2-trichloromethylquinazoline **Hits A** and **B**. Thus, we have prepared the matrices followed by allowing them to contact with the parasite extracts, and finally, the plasmodial proteins were identified as the possible targets of **Hit A**.

Table 1. Structures of antiplasmodial quinazoline Hits A and B and properties of Hit A



Antiparasitic activity profile	EC_{50} (W2 or K1) <i>P. falciparum</i> (μM)	0.4
	EC_{50} <i>T. gondii</i> (μM)	> 50
	EC_{50} <i>L. donovani</i> promastigotes (μM)	> 50
	EC_{50} <i>T. vaginalis</i> (μM)	> 50
In vitro toxicity	CC_{50} HepG2 (μM)	16
	Ames test @ 1 mM (<i>S. typhimurium</i> TA97a, TA98, TA100, TA102)	Negative
Lipophilicity	Dlog @ pH 7.4	3.6

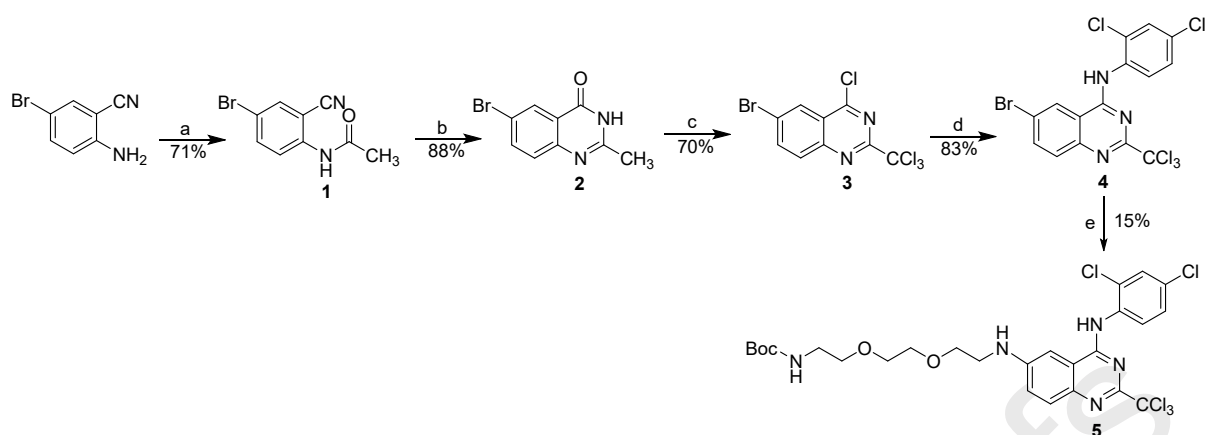
<i>In vitro</i> pharmacokinetic parameters	Female Mouse Plasma half-life (h)	> 6
	Mouse liver microsomal half-life (min)	18
<i>Tested mechanisms of action</i>	Heme crystallization inhibition (chloroquine)	Negative
	Production of radical intermediates (artemisinin)	Negative
	<i>Pf</i> DHFR Inhibition (proguanil)	Negative
<i>Erythrocytic stage targeted</i>	Early trophozoites	Maximal effect @ 8 < t < 16 h

Results and discussion

1. Preparation of the affinity matrix

Two different drug-linked affinity matrices were obtained, by immobilizing **Hit A** or **Hit B** on solid supports. In a first step, linkers were introduced at various positions on the hits, to avoid interference with target interaction, looking for a linked-hit derivative preserving *in vitro* antiplasmodial properties of the parent hit: C6 and C7 positions for **Hit A**, and C3' position for **Hit B** were selected for the study. Three multi-step synthesis pathways allowed to obtain 4 linked-hits.

First, molecule **5**, corresponding to linked-**Hit A** bearing a PEG-type linker at C6 position, was obtained in 5 steps (Scheme 1). Briefly, 2-amino-5-bromobenzonitrile was *N*-acetylated under microwave irradiation for 6 min, reacting with acetyl chloride in the presence of pyridine in DMF to afford **1** in 71% yield. Then, nitrile **1** was cyclized into quinazolinone **2** by reaction with hydrogen peroxide and sodium hydroxide in ethanol/water for 5 min at 50°C (88%). Methylquinazolinone **2** was tetrachlorinated into 2-trichloromethylquinazoline **3** by reacting with a mixture of phosphorus pentachloride and phosphorus oxychloride in a sealed vial under microwave irradiation for 30 min (70%). *Via* a S_NAr reaction between **3** and 2,4-dichloroaniline in isopropanol at 80°C, compound **4** was obtained in 83% yield. Finally, a Buchwald-Hartwig reaction between brominated compound **4** and *tert*-butyl{2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate led to expected product **5** in a low 15% yield.



a) Acetyl chloride 2 equiv., pyridine 3 equiv., DMF, MW, 150 W, 50 °C, 6 min ; b) H₂O₂ 50 equiv., NaOH 1.6 equiv., EtOH/H₂O (1:1), 50 °C, 5 min ; c) PCl₅ 6 equiv., POCl₃ 12 equiv., MW, 800 W, 110 °C, 30 min ; d) 2,4-dichloroaniline 2 equiv., *i*-PrOH, 80 °C, 72 h ; e) *tert*-butyl{2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate 1.5 equiv., Pd(OAc)₂ 5% mol, Xantphos 10% mol, K₂CO₃ 3 equiv., dioxane, N₂, 90 °C, 72 h.

Scheme 1. Synthesis of compound 5: linked-Hit A at C6 position

Compound **11** (linked-Hit A), bearing the same PEG-type linker at C7 position was obtained in 6 steps (Scheme 2). Briefly, 2-amino-4-methylbenzonitrile was *N*-acylated with chloroacetyl chloride in DMF under microwave irradiation at 50°C for 5 min, leading to **6** in 91% yield. Nitrile **6** was then cyclized into quinazolinone **7** by reacting with a mixture of hydrogen peroxide and sodium hydroxide in a mixture of ethanol and water at 50°C for 5 min (82%). Compound **7** was then trichlorinated by heating in a mixture of phosphorus pentachloride and phosphorus oxychloride at 110°C for 30 min in a sealed vial under microwave irradiation, giving the quinazoline **8** in 93% yield. Free radical bromination of **8** with *N*-bromosuccinimide (NBS) and benzoyl peroxide in carbon tetrachloride under light irradiation at 75°C for 30 h led to compound **9** in 38% yield. The S_NAr reaction between **9** and 2,4-dichloroaniline (0.8 equiv. to avoid S_N2 reaction on the bromomethyl group), in the presence of catalytic amount of HCl in isopropanol at 70°C under microwave irradiation afforded compound **10** in 60% yield. Finally, bromomethylated compound **9** was reacted with *tert*-butyl{2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate in the presence of triethylamine in DMF at RT, providing expected compound **11** in 67% yield. To study the influence of linker length, we also synthesized compound **12** from bromomethyl **10**, using the same S_N2 reaction with another mono-*N*-protected diamine PEG-derivative (Scheme 2).

An important point is also to check that introduction of the linker does not interfere with the biological activity

of the considered drug (or that at least some of its activity is retained). The position where the linker can be introduced, while maintaining the biological activity of the drugs, can be determined in two different ways.

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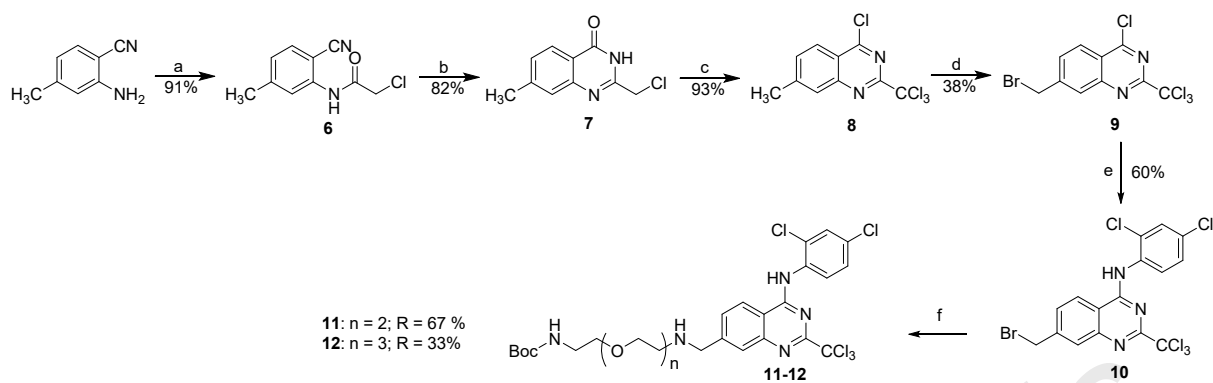
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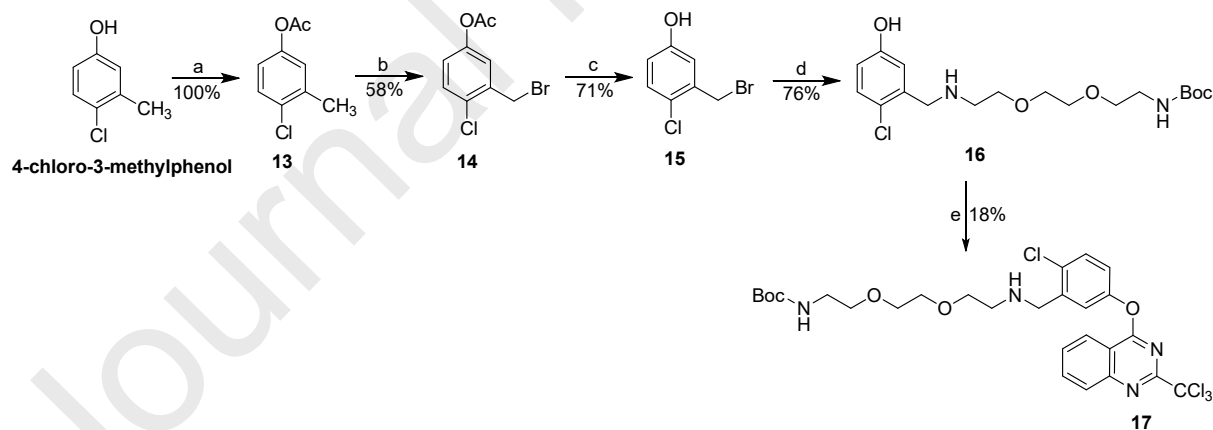
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a) Chloroacetyl chloride 2 equiv., pyridine 3 equiv., DMF, MW, 150 W, 50 °C, 5 min; b) H₂O₂ 50 equiv., NaOH 1.6 equiv., EtOH/H₂O (2:1), 50 °C, 5 min; c) PCl₅ 4 equiv., POCl₃ 10 equiv., MW, 800 W, 110 °C, 30 min; d) NBS 1.2 equiv., benzoyl peroxide 0.1 equiv., CCl₄, hv, 75 °C, 30 h e) 2,4-dichloroaniline 0.8 equiv., HCl 37% cat., *i*-PrOH, MW, 800 W, 70 °C, 2 h; f) *tert*-butyl{2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate or *tert*-butyl(3-[2-[2-(3-aminopropoxy)ethoxy]ethoxy]propyl)carbamate 2 equiv., NEt₃ 1.5 equiv. DMF, RT, 30 h.

Scheme 2. Synthesis of compounds 11 and 12: Hit A derivatives bearing a linker at C7 position

Moreover, a PEG-type linker was attached at C3' position of **Hit B**, to afford compound **17** in 5 steps (Scheme 3). The OH group of 4-chloro-3-methylphenol was protected by acid catalysed *O*-acetylation with acetic anhydride, affording **13** in quantitative yield. Compound **13** was then brominated by reaction with NBS/AIBN in CCl₄, under light irradiation at 75 °C for 2 h, leading to **14** in 58% yield. Compound **14** was deprotected into phenol **15** with ammonium acetate in methanol at RT (71%). By reacting bromomethylated compound **15** with *tert*-butyl{2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate, compound **16** was isolated in 76% yield. Finally, the phenolate conjugate base of phenol **16**, formed with NaH in dry DMF, was reacted with 4-chloro-2-trichloromethylquinazoline in DMF at RT, leading to S_NAr product **17** in 18% yield.



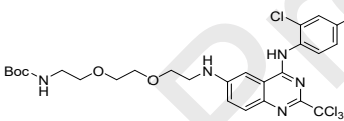
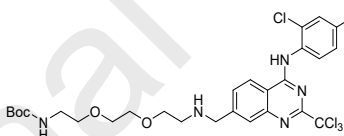
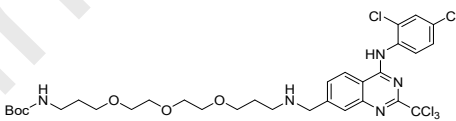
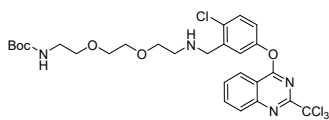
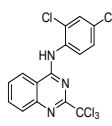
a) Acetic anhydride 1.3 equiv., H₂SO₄ conc. cat., 60 °C, 2 h. b) NBS 1 equiv., AIBN 0.1 equiv., CCl₄, hv, 75 °C, 2 h. c) NH₄OAc 10 equiv., MeOH, RT, 12 h. d) *tert*-butyl{2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate 1.3 equiv., NEt₃ 1 equiv. THF, RT, 12 h. e) 1) NaH 1.3 equiv., DMF, sealed vial, RT, N₂, 30 min; 2) 4-chloro-2-trichloromethylquinazoline 1 equiv., DMF, RT, N₂, 9 h.

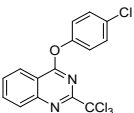
Scheme 3. Synthesis of compound 17: linked-Hit B at C3' position

To evaluate the effect of the linker and its position on the hit scaffolds toward antiplasmodial activity, the four linked-Hits (**5**, **11**, **12**, **17**) were tested on both K1 *P. falciparum* parasites and HepG2 cell cultures, determining their 50% efficacy concentrations (EC₅₀), their 50% cytotoxic concentrations (CC₅₀), and their corresponding selectivity indices (SI = CC₅₀/EC₅₀), as presented in Table 2. Biological

results indicated that compound **11** possessed similar characteristics to **Hit A** and was the best candidate for anchoring to a solid support. Compounds **5**, **12** and **17** were either more cytotoxic or less selective than the corresponding antiplasmodial **Hits A** and **B**.

Table 2. *In vitro* biological evaluation of linked-Hits

N°	Formula	K1 <i>P. falciparum</i>	HepG2	SI
		EC ₅₀ (μM)	CC ₅₀ (μM)	
5		3	5	1.7
11		0.6	19	32
12		0.15	3.1	21
17		0.35	8.5	24
Hit A		0.4	16	40

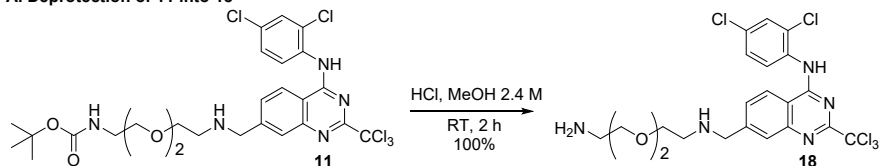
Hit B		1.1	50	45
Reference drug-compounds	Doxycycline	5	20	4
	Chloroquine	0.5	30	60
	Artesunate	0.004		
	Doxorubicin		0.2	

To generate matrices for affinity chromatography, two solid supports were considered: Sepharose® beads and Synphase lanterns®. Sepharose® beads have an excellent biocompatibility and are the most frequently used matrices for target fishing experiments [23]. Nevertheless, as the matrix is in the form of a gel, its handling requires washing and centrifugation steps, and the use of a bacteriostatic preservative (NaN₃). An alternative to these constraints is the use of lanterns [24]. Traditionally employed for solid-phase organic synthesis [25], lanterns are macroscopic modular solid substrates, that have the advantage of avoiding the washing/centrifugation steps. Moreover, their conservation in a dry form limits potential biological contamination. The polyamide lanterns, functionalized by hydroxyphenylmethoxy groups (called HMP Lanterns), also exhibit a good biocompatibility.

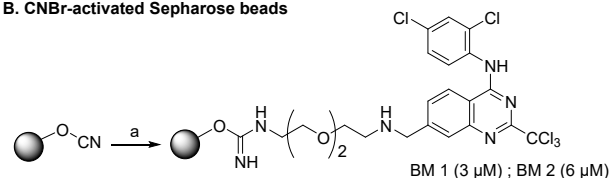
Before anchoring on the Sepharose® beads, compound **11** was deprotected with 7 N HCl in methanol at RT, to obtain the amine derivative **18** in quantitative yield (Scheme 4A). Then, bead matrix 1 (**BM1**) and bead matrix 2 (**BM2**) were obtained by anchoring **18** on the sepharose beads, allowing cyanogen bromide-activated beads to react with a solution of **18** (at the concentration of 3 or 6 μM) in a DMF/water (1/1) mixture buffered at pH = 8 for 2 h (Scheme 4B). Negative-control beads were also prepared by coupling sepharose beads with ethanolamine.

Lantern matrices were obtained by adapting a protocol described by Cosquer *et al.* [26]. HMP lanterns were esterified with chloroacetyl chloride yielding **LM1**. Then, **LM1** was reacted with a di-amino-PEG-type linker, leading to **LM2** which was finally reacted with the brominated molecule **10** in the presence of triethylamine, to afford **LM3** (Scheme 4C).

A. Deprotection of 11 into 18

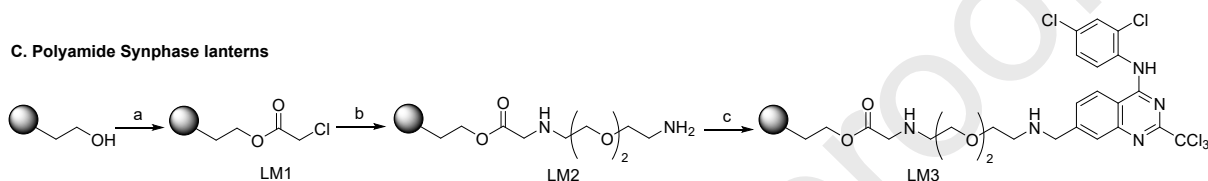


B. CNBr-activated Sepharose beads



a) **18** @ 3 μM or 6 μM , DMF/H₂O 1:1, pH = 8, RT, 2 h

C. Polyamide Synphase lanterns



a) Chloroacetyl chloride 0.2 M, pyridine 0.2 M, CH₂Cl₂ RT, 2 h. b) 2,2'-(ethane-1,2-diylbis(oxy))diethylamine 0.4 M, DMSO, RT, 2 h.
 c) **10** @ 0.2 M, DMF, RT, 12 h

Scheme 4. Synthesis of matrices: A) acidic deprotection of tBu-carbamate 11 into amine 18, B) reaction of 18 with Sepharose beads to generate a bead matrix (BM), C) synthesis of lantern matrix (LM) from bromomethyl compound 10

2. Affinity chromatography of *P. falciparum* extracts on hit A-matrices

Crude extracts of *P. falciparum* were obtained from a *P. falciparum* culture using two different lysis buffers (Py+NP40 or M-PER™, Thermo Scientific). Then, crude parasite extracts solubilized in homogenization buffer were incubated at 4 °C for 30 min under constant rotation, with control beads (without linked compound), **BM1** and **BM2**. After extensive washing of the bead matrix with bead buffer, Laemmli sample buffer was added to the beads, and the bound proteins were eluted with electrophoresis sample buffer and resolved by SDS-PAGE electrophoresis, followed by silver staining. The use of **BM2** and M-PER lysis buffer allowed to identify five protein bands (B1-B5), which were excised from the gel for identification by mass tandem spectrometry sequencing (Figure 1). The same protocol was applied with **LM3**, without needing centrifugation steps. Two supplementary protein bands (B6 & B7) were then excised from the SDS-PAGE for identification (Figure 2).

Analysis of the 7 excised bands by mass spectrometry revealed two parasitic proteins that bind specifically to the **BM2** matrix, in comparison with the ethanolamine negative control bead matrix: B2 = *PfPYK-1* and B5 = *PfRab6* (Figure 1). B1, B3, B4, B6 and B7 (Figures 1 and 2) corresponded to a binding of the matrix **BM2** or **LM3** with proteins which could not be identified as parasitic proteins.

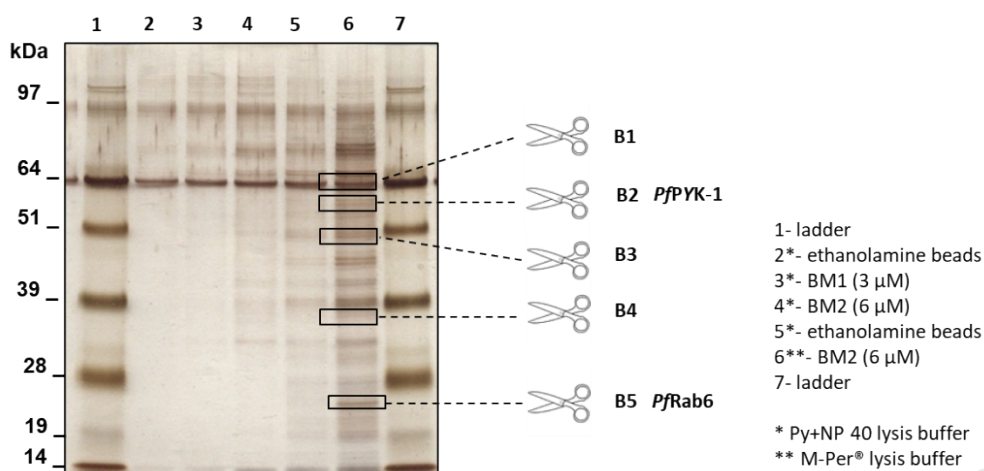


Figure 1. Protein interacting with hit A-bead matrices BM2 from a *P. falciparum* extract. The bound proteins were resolved by SDS-PAGE, followed by silver staining. Proteins B1 to B5 were further processed for microsequencing. B2 was identified as *PfPYK-1* and B5 was identified as *PfRab6*.

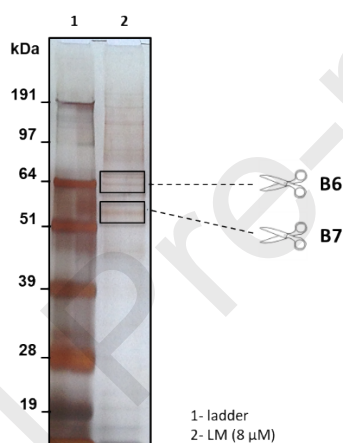


Figure 2. Protein interacting with Hit A-lantern matrix LM3 from a *P. falciparum* extract. The bound proteins were resolved by SDS-PAGE, followed by silver staining. Proteins B6 and B7 were further processed for microsequencing.

The *P. falciparum* small Rab GTPase *PfRab6* (PlasmoDB accession number PF3D7_1144900) is a 207 amino acid protein, expressed throughout the erythrocyte cycle, with highest levels at the ookinete stage. It is one of the 11 members of the *P. falciparum* Rab GTPase family, known as regulators of vesicular trafficking and endocytosis in eukaryotes. *PfRab-6* is strongly associated with the Golgi apparatus, but does not appear to be required for parasite development in erythrocytes. [27-29] *PfRab-6* structure (PDB ID : 1D5C) was also the first *PfRab* structure to be solved, complexed to GDP [30]. Interestingly, *PfRab6* was one of the proteins co-immunoprecipitated with Kelch protein K13 in a recent study [31]. To our knowledge, no *PfRab6* specific inhibitor has yet been identified.

The *P. falciparum* pyruvate kinase 1 *PfPYK-1* (PlasmoDB accession number PF3D7_0626800) is a 511 amino acid cytosolic protein, consistently expressed throughout the erythrocytic cycle. *PfPYK-1* belongs to the cluster 1 of the pyruvate kinases phylogenetic analysis [32]. In the genome-wide piggyback transposon saturation study published by the Adams laboratory [33], *PfPYK-1* displayed a very low MIS (mutagenesis index score) of 0.147, suggesting essentiality for asexual proliferation. Indeed, *PfPYK-1* was directly shown to be essential for parasite development, which relies on the protein for regulating glycolytic flux and ATP production [32, 34]. The *PfPYK-1* structure was recently solved by X ray crystallography, complexed with substrate analogues, highlighting an inactive T-state (PDB ID: 3KH) and an active R-state (PDB ID:6KSH). The authors also demonstrated the inhibition of *PfPYK-1*

enzymatic activity with oxalate ($IC_{50} = 150 \pm 11 \mu\text{M}$) or anti-trypanosome drug, suramin ($IC_{50} = 128 \pm 14 \mu\text{M}$) [35].

Both *PfRab6* and *PfPYK-1* have been identified as enzymes binding to purine derivatives, their respective natural ligands being guanosine triphosphate (GTP) and adenosine diphosphate (ADP). The antiplasmodial mechanism of action of 4-anilinoquinazoline **Hit A** may involve competitive inhibition of these two enzymes in *P. falciparum*, making **Hit A** a potential purine analogue inhibitor chemotype for the conception of new antimalarial derivatives presenting novel mechanisms of action.

Conclusion

A target fishing strategy was followed to investigate the mechanism of action of previously identified trichloromethyl-containing antiplasmodial quinazoline hits. The first step involved the introduction of a PEG-type linker at various positions on the hit scaffolds, looking for a linked-Hit preserving the antiplasmodial properties of the parent hit. Thus, a derivative of 4-anilinoquinazoline **Hit A**, bearing a PEG-type linker at position 7 was identified as a good candidate and was then anchored on two solid supports: sepharose beads and polyamide lanterns. Using an affinity chromatography approach, after contact with a *P. falciparum* extract followed by SDS-PAGE and mass spectrometry analysis, a Sepharose® matrix allowed the identification of two *P. falciparum* proteins, *PfRab6* and *PfPYK-1*, as probable targets of **Hit A**. As both these two enzymes bind to GTP or ADP, **Hit A** could act as a purine analogue, allowing competitive inhibition of *PfRab6* and *PfPYK-1*, making **Hit A** an original chemotype for the conception of new antimalarials with novel mechanisms of action.

Experimental section

1. Chemistry: Preparation of drug-linked affinity matrices

1.1. General

Commercial reagents were used as received without additional purification. Melting points were determined on a Köflier bench and are uncorrected. Elemental analysis and HRMS were carried out at the Spectropole, Faculté des Sciences et Techniques de Saint-Jérôme, Marseille, France. NMR spectra were recorded on a Bruker ARX 200 spectrometer or a Bruker AV 250 spectrometer at the Faculté de Pharmacie de Marseille or a BRUKER Avance III nanobay 400 at the Spectropole, Faculté des Sciences et Techniques de Saint-Jérôme, Marseille (^1H NMR: 200, 250 or 400 MHz, ^{13}C NMR: 50, 63 or 100 MHz). NMR references were the following: ^1H : CHCl_3 $\delta = 7.26$, $\text{DMSO-}d_6$ $\delta = 2.50$ and ^{13}C : CHCl_3 $\delta = 76.9$, $\text{DMSO-}d_6$ $\delta = 39.5$. Solvents were dried by conventional methods. The following adsorbent was used for column chromatography: silica gel 60 (Merck, particle size 0.063-0.200 mm, 70-230 mesh ASTM). TLC was performed on 5 cm X 10 cm aluminium plates coated with silica gel 60F-254 (Merck) in an appropriate eluent. Visualization was made with ultraviolet light (234 nm). HRMS spectra were recorded on QStar Elite (Applied Biosystems SCIEX) spectrometer. PEG was the matrix for HRMS. The experimental exact mass was given for the ion which has the maximum isotopic abundance. Purity of synthesized compounds was checked with LC-MS analyses which were realized at the Faculté de Pharmacie de Marseille with a Thermo Scientific Accela High Speed LC System® coupled with a single quadrupole mass spectrometer Thermo MSQ Plus®. The RPHPLC column used is a Thermo Hypersil Gold® 50 X 2.1 mm (C18 bounded), with particles of 1.9 mm diameter. The volume of sample injected on the column was 1 mL. The chromatographic analysis, total duration of 8 min, is made with the gradient of following solvents: $t = 0$ min, water/methanol 50/50; $0 < t < 4$ min, linear increase in the proportion of methanol to a ratio water/methanol 5/95; $4 < t < 6$ min, water/methanol 5/95; $6 < t < 7$ min, linear decrease in the proportion of methanol to return to a ratio 50/50 water/methanol; $6 < t < 7$ min, water/methanol 50/50. The water used was buffered with 5 mM ammonium acetate. The retention times (t_R) of the molecules analyzed are indicated in min.

1.2. Organic synthesis

1.2.1. *N*-(4-bromo-2-cyanophenyl)acetamide (**1**)

As previously described [36], to a mixture of 2-amino-5-bromobenzonitrile (1 equiv., 5.08 mmol) in pyridine (3 equiv., 15.22 mmol), acetylchloride (2 equiv., 10.16 mmol) in DMF (40 mL) was added dropwise at 0 °C. The reaction mixture was then irradiated in a microwave oven at 50 °C, for 6 min. at a power of 150 W. After cooling, the reaction mixture was poured onto cold water (100 mL). The precipitate formed was filtered, washed with water (3 × 20 mL) and dried. Silica gel column chromatography (AcOEt/CH₂Cl₂ 1/9) gave white solid, yield 71%. mp: 165 °C (lit. 165 °C) [37], MW: 239.07 g/mol. ¹H NMR (CDCl₃, 200 MHz): δ (ppm) 2.26 (3H, s), 7.65-7.70 (3H, m), 8.32-8.37 (1H, m). ¹³C NMR (CDCl₃, 50 MHz): δ (ppm) 24.8, 103.3, 115.0, 116.2, 127.7, 134.3, 137.3, 139.6, 168.5. LC/MS (APCI⁺): tr= 1.07 min, m/z: 239.3 [M+H]⁺. Anal. calcd. for C₉H₇BrN₂O: C, 45.22; H, 2.95; N, 11.72. Found: C, 45.26; H, 3.06; N, 11.67.

1.2.2. 6-Bromo-2-methylquinazolin-4-(3*H*)-one (**2**)

As previously described, [38] to a mixture of **1** (1 equiv., 2.3 mmol) in ethanol/H₂O (1/1) at 0 °C, NaOH pellets (1.6 equiv., 3.68 mmol) and H₂O₂ solution (50 equiv., 115 mmol) were added. After 15 min of stirring at rt, the reaction mixture was heated until a homogeneous solution was obtained. After cooling, ethanol was removed under vacuum and 250 mL of water were added. The resulting mixture was heated at 50 °C for 5 min. After cooling, a solution of sulphuric acid (1M) was added up to pH=4. The precipitate formed was filtered and oven-dried to give a white solid, yield: 88%, mp: 297 °C (lit. 298 °C) [38], MW: 239.07 g/mol. ¹H NMR (DMSO-*d*₆, 200 MHz): δ (ppm) 2.33 (3H, s), 7.50 (1H, d, *J*= 8.7 Hz), 7.89 (1H, dd, *J*= 2.4 Hz and *J*= 8.7 Hz), 8.12 (1H, d, *J*= 2.4 Hz) one non-visible proton in these experimental conditions. ¹³C NMR (DMSO-*d*₆, 50 MHz): δ (ppm) 21.7, 118.3, 122.4, 128.0, 129.1, 137.2, 148.0, 155.3, 160.8. LC/MS (ESI⁺): tr= 1.45 min, m/z: 239.1/241.1 [M+H]⁺. Anal. calcd. for C₉H₇BrN₂O: C, 45.22; H, 2.95; N, 11.72. Found: C, 45.74; H, 3.13; N, 11.23.

1.2.3. 6-Bromo-4-chloro-2-trichloromethylquinazoline (**3**)

Compound **2** (1 equiv., 10 mmol) was added to a mixture of PCl₅ (6 equiv., 60.2 mmol) and POCl₃ (12 equiv., 120.4 mmol) in a vial at 0 °C. The vial was then heated in a microwave oven, irradiating with 800 W, heating to 110 °C for 30 min. After cooling, the mixture was poured onto a saturated sodium carbonate solution and extracted with CH₂Cl₂ (3 x 100 mL). The crude product obtained was then purified by silica gel column chromatography (Petroleum Ether/CH₂Cl₂ 1/1) to afford a white solid, yield: 70%, mp: 182 °C, MW: 360.85 g/mol. ¹H NMR (CDCl₃, 200 MHz): δ (ppm) 8.10-8.13 (2H, m), 8.50-8.52 (1H, m). ¹³C NMR (CDCl₃, 50 MHz): δ (ppm) 95.7, 123.9, 125.0, 128.2, 131.2, 139.6, 148.9, 160.1, 162.8. LC/MS (ESI⁺): tr= 4.98 min, m/z: 301.0/302.6/304.1 [M+H]⁺. Anal. calcd. for C₉H₃BrCl₄N₂: C, 29.96; H, 0.84; N, 7.76. Found: C, 31.30; H, 1.03; N, 7.60.

1.2.4. 6-Bromo-*N*-(2,4-dichlorophenyl)-2-trichloromethylquinazolin-4-amine (**4**)

A solution of **3** (1 equiv., 13.8 mmol) and 2,4-dichloroaniline (2 equiv., 27.6 mmol) in isopropanol (100 mL) was heated at 80 °C for 72 h. After cooling, the reaction mixture was extracted with CH₂Cl₂ (3 x 50 mL) and the organic layer was washed with water (3 x 100 mL), dried over Na₂SO₄, filtered, and evaporated. An orange residue was obtained then washed with CH₂Cl₂ (10 mL) to afford a pale yellow solid, yield: 83%, mp: 232 °C, MW: 486.41 g/mol. ¹H NMR (CDCl₃, 200 MHz): δ (ppm) 7.50-7.55 (1H, m), 7.67-7.72 (1H, m), 7.77-7.91 (2H, m), 8.09-8.14 (1H, m), 8.90 (1H, s), 10.45 (1H, s). ¹³C NMR (CDCl₃, 50 MHz): δ (ppm) 97.6, 115.1, 121.1, 126.0, 127.9, 129.4, 130.3, 130.8, 131.5, 131.6, 134.3, 137.5, 148.1, 159.0, 160.5. LC/MS (ESI⁺): tr= 5.63 min, m/z: 485.56/487.67/489.30 [M+H]⁺. Anal. calcd. for C₁₅H₇BrCl₅N₃: C, 37.04; H, 1.45; N, 8.64. Found: C, 37.25; H, 1.40; N, 8.50.

1.2.5. *Tert*-butyl-(2-{2-[2-({4-[(2,4-dichlorophenyl)amino]-2-trichloromethyl-quinazolin-6-yl}amino)ethoxy]ethoxy} ethyl)carbamate (**5**)

In a sealed vial, **4** (1 equiv., 1.03 mmol), palladium acetate (0.05 equiv., 0.05 mmol), potassium carbonate (3 equiv., 3.09 mmol) and Xantphos (0.1 equiv., 0.10 mmol) were introduced and placed under nitrogen atmosphere. A solution of *tert*-butyl{2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate (1.5 equiv., 1.55 mmol) in 20 mL of dioxane was added and the reaction mixture was heated 72 h at 90 °C. After cooling, water was added and the mixture was extracted with CH₂Cl₂ (3 x 20 mL). The organic layer was washed with water (3 x 50 mL), dried over Na₂SO₄, filtered, and evaporated. The crude residue was purified by silica gel column chromatography (MeOH/CH₂Cl₂/NH₃ 5.8/94/0.2) to give compound **5** as a yellow solid. MW: 653.81 g/mol. Yield: 15%. Mp: 70°C. ¹H NMR (CDCl₃, 200 MHz): δ (ppm) 1.41 (9H, s), 3.26-3.33 (2H, m), 3.51-3.55 (2H, m), 3.63-3.76 (8H, m), 5.04 (1H, ls), 7.34-7.39 (1H, m), 7.47-7.48 (1H, m), 7.95 (1H, s), 8.13 (1H, s), 8.27-8.31 (1H, m), 8.64-8.68 (1H, m), 2 non-visible protons in these experimental conditions. ¹³C NMR (CDCl₃, 50 MHz): δ (ppm) 28.4 (3), 39.7, 40.4, 69.9, 70.2, 70.3 (2), 95.6, 116.3, 122.2, 123.3, 124.1, 125.2, 128.1, 129.1, 129.7, 131.8, 133.3, 137.3, 148.8, 153.4, 156.0, 156.4, 163.0. LC/MS (ESI⁺): tr= 4.48 min, HRMS *m/z* [M+H]⁺ calcd for C₂₆H₃₀Cl₃N₅O₄: 651.0740 Da, found: 651.0744 Da.

1.2.6. 2-Chloro-*N*-(2-cyano-5-methylphenyl)-acetamide (**6**), 2-chloromethyl-7-methylquinazolin-4(3*H*)-one (**7**), and 4-chloro-7-methyl-2-trichloromethylquinazoline (**8**) were obtained using previously described procedures. [39, 40]

1.2.7. 7-Bromomethyl-4-chloro-2-trichloromethylquinazoline (**9**)

A mixture of **8** (1 equiv., 3.38 mmol), *N*-bromosuccinimide (1.2 equiv., 4.05 mmol), benzoyl peroxide (0.1 eq, 0.34 mmol) and CCl₄ (20 mL) was stirred at 75 °C under light irradiation for 30 h. Water was then added (100 mL) and the mixture was extracted with CH₂Cl₂ (3 x 20 mL). The organic layer was washed with water (3 x 50 mL), dried over Na₂SO₄, filtered, and evaporated. The crude residue was purified by silica gel column chromatography (toluene/cyclohexane 1/4) to afford a white solid, yield: 38%, mp: 118°C, MW: 374.88 g/mol. ¹H NMR (CDCl₃, 200 MHz): δ (ppm) 4.66 (2H, s), 7.85 (1H, dd, *J*= 8.6 Hz and *J*= 1.2 Hz), 8.19 (1H, d, *J*= 1.2 Hz), 8.32 (1H, d, *J*= 8.6 Hz). ¹³C NMR (CDCl₃, 50 MHz): δ (ppm) 31.0, 95.8, 122.4, 126.6, 129.1, 131.6, 146.2, 150.3, 160.4, 163.7. LC/MS (ESI⁺): tr= 4.81 min, HRMS *m/z* [M+H]⁺ calcd for C₁₀H₅BrCl₄N₂: 372.84630 Da, found: 372.84658 Da.

1.2.8. 7-Bromomethyl-*N*-(2,4-dichlorophenyl)-2-trichloromethylquinazoline (**10**)

To a solution of **9** (1 equiv., 2.67 mmol) and 2,4-dichloroaniline (0.8 equiv., 2.13 mmol) in isopropanol (25 mL), few drops of HCl 37% were added. The reaction mixture was placed in the microwave oven irradiating with 800 W heating to 70 °C for 2 h. After cooling, water was added (100 mL) and the mixture was extracted with CH₂Cl₂ (3 x 50 mL). The organic layer was washed with water, dried over Na₂SO₄, filtered, and evaporated. The crude residue was washed with CH₂Cl₂ (20 mL) to afford a white solid, yield: 60%, mp: 221°C, MW: 500.43 g/mol. ¹H NMR (CDCl₃, 200 MHz): δ (ppm) 4.77 (2H, s), 7.38-7.51 (2H, m), 7.76 (1H, dd, *J*= 8.4 Hz and *J*= 1.6 Hz), 7.94-8.00 (1H, m), 8.12 (1H, s), 8.34 (1H, s), 9.07 (1H, d, *J*= 8.4 Hz). ¹³C NMR (CDCl₃, 50 MHz): δ (ppm) 45.0, 97.3,

113.6, 120.9, 122.9, 123.8, 128.3, 128.8, 129.0, 129.1, 129.5, 133.3, 143.8, 149.8, 157.0, 161.2. LC/MS (ESI⁺): *tr*= 5.42 min, *m/z*: 497.42/499.57/501.62/503.61/505.54 [M+H]⁺. HRMS *m/z* [M+Na]⁺ calcd for C₁₆H₉BrCl₅N₂: 519.83147 Da, found: 519.83139 Da.

1.2.9. Synthesis of the linked-hits (**11**) and (**12**), bearing PEG-type linkers at position 7 of the quinazoline ring

General procedure

To a solution of **10** (1 equiv., 1 mmol) in DMF (20 mL), NEt₃ (1.5 equiv., 1.5 mmol) and appropriated linker (2 equiv.) were added. The reaction mixture was stirred at rt for 30 h. Water was then added (100 mL) and the mixture was extracted three times with CH₂Cl₂ (3 x 20 mL). The organic layer was washed with water (3 x 100 mL), dried over Na₂SO₄, filtered, and evaporated. The crude residue was purified by silica gel column chromatography (CH₂Cl₂/MeOH/NH₃ 90/9.9/0.1) to afford **11** or **12**.

Tert-butyl-2-([2-[2-([4-[2,4-dichlorophenylamino]-2-(trichloromethylquinazolin-7-yl)methylamino)ethoxy]ethoxy]ethylcarbamate (**11**). Linker: *tert*-butyl{2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate. MW: 667.84 g/mol. Pale yellow oil. Yield: 67%. ¹H NMR (CDCl₃, 200 MHz): δ (ppm) 1.42 (9H, s), 2.13 (2H, ls), 2.85 (2H, t, *J*= 4.9 Hz), 3.32 (2H, q, *J*= 4.9 Hz), 3.52-3.58 (2H, m), 3.63-3.65 (6H, m), 4.07 (2H, s), 7.40 (1H, dd, *J*= 2.4 Hz and *J*= 9 Hz), 7.48 (1H, d, *J*= 2.4 Hz), 7.75-7.79 (1H, m), 7.91-7.95 (1H, m), 8.06 (1H, s), 8.33 (1H, s), 9.07 (1H, d, *J*= 9 Hz). ¹³C NMR (CDCl₃, 50 MHz): δ (ppm) 28.4 (3CH₃), 40.3, 48.5, 53.1, 70.2 (2CH₂), 70.3 (2CH₂), 79.3, 97.5, 113.0, 120.2, 122.8, 123.7, 128.2, 128.7, 128.8, 129.2, 133.5, 140.3, 147.2, 149.8, 156.0, 157.0, 160.8. LC/MS (ESI⁺): *tr*= 5.42 min, *m/z*: 665.72/667.78/669.74 [M+H]⁺. HRMS *m/z* [M+H]⁺ calcd for C₂₇H₃₂Cl₅N₅O₄: 666.09697 Da, found: 666.09680 Da.

Tert-butyl-1-[4-([2,4-dichlorophenyl]amino)-2-trichloromethylquinazolin-7-yl]-6,9,12-trioxa-2-azapentadecan-15-yl]carbamate (**12**). Linker: *tert*-butyl(3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}propyl)carbamate. MW: 739.94 g/mol. Pale yellow oil. Yield: 33%. ¹H NMR (CDCl₃, 200 MHz): δ (ppm) 1.39 (9H, s), 1.67-1.73 (2H, m), 1.95-2.01 (2H, m), 3.15-3.18 (2H, m), 3.45-3.59 (16H, m), 4.29 (1H, ls), 5.04 (1H, ls), 7.30-7.43 (2H, m), 7.86-8.03 (3H, m), 8.33-8.42 (1H, m), 8.84-8.96 (1H, m). ¹³C NMR (CDCl₃, 50 MHz): δ (ppm) 28.4 (3CH₃), 29.6, 38.3 (2CH₂), 46.5, 51.9, 69.28, 69.34, 70.0, 70.1, 70.3, 70.4, 97.4, 113.0, 113.4, 121.0, 123.3, 124.4, 128.0, 128.7 (2CH), 129.2, 129.6, 133.3, 145.3, 149.5, 156.0, 157.1, 160.9. LC/MS (ESI⁺): *tr*= 5.48 min, *m/z*: 736.84/738.9/740.7 [M+H]⁺. HRMS *m/z* [M+H]⁺ calcd for C₃₁H₄₀Cl₅N₅O₅: 737.1472 Da, found: 737.1475 Da.

1.2.10. 4-Chloro-3-methylphenylacetate (**13**)

As a previously described procedure, [41] a mixture of 4-chloro-3-methylphenol (1 equiv., 70.3 mmol), acetic anhydride (1.3 equiv., 91.4 mmol) and few drops of H₂SO₄ conc was heated to 60 °C for 2 h. After cooling, water was added (50 mL) and the reaction mixture is stirred for 1 h. The reaction mixture is then extracted with ethyl acetate (2 x 50 mL), and the organic layer is dried over Na₂SO₄, filtered, and evaporated to afford a colorless oil,

$C_9H_9ClO_2$ MW: 184.62 g/mol, yield: 100% [42]. 1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 2.28 (3H, s), 2.36 (3H, s), 6.85-6.99 (2H, m), 7.33 (1H, d, $J=8.6$ Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 19.9, 20.8, 120.1, 123.8, 129.5, 131.1, 137.1, 148.8, 169.1. LC/MS (ESI⁺): $t_r=3.07$ min, m/z : 202.31/204.33 [$M+NH_4$]⁺.

1.2.11. 3-Bromomethyl-4-chlorophenylacetate (**14**)

As a previously described procedure, [43] a mixture of **13** (1 equiv., 27 mmol), *N*-bromosuccinimide (1 equiv., 27 mmol), AIBN (0.1 equiv., 3 mmol) and CCl_4 was stirred at 75 °C under light irradiation for 2 h. Water was then added (30 mL) and the mixture was extracted three times CH_2Cl_2 (3 x 20 mL). The organic layer was washed with water (20 mL), dried over Na_2SO_4 , filtered, and evaporated. The crude residue was purified by silica gel column chromatography (toluene/cyclohexane 1/1) to afford a white solid, yield: 58%, mp: 45°C (lit. not described) [44], $C_9H_8BrClO_2$ MW: 263.52 g/mol. 1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 2.30 (3H, s), 4.55 (2H, s), 7.03 (1H, dd, $J=2.8$ Hz and $J=8.6$ Hz), 7.20-7.22 (1H, m), 7.39 (1H, d, $J=8.6$ Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 21.0, 29.8, 123.2, 124.2, 130.7, 131.0, 136.4, 149.2, 169.0. LC/MS (ESI⁺): $t_r=3.07$ min, m/z : 280.11/282.14/284.12 [$M+NH_4$]⁺.

1.2.12. 3-Bromomethyl-4-chlorophenol (**15**)

A mixture of **14** (1 equiv., 1.9 mmol) and ammonium acetate (10 equiv., 19 mmol) in methanol (25 mL) was stirred at rt for 12h. Water was then added (50 mL), and the reaction mixture was extracted three times with CH_2Cl_2 (3 x 20 mL). The organic layer was washed with water (20 mL), dried over Na_2SO_4 , filtered, and evaporated. The crude residue was purified by silica gel column chromatography (CH_2Cl_2) to afford a white solid, yield: 71%, mp: 66°C (lit. not described) [45], MW: 221.48 g/mol. 1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 4.51 (2H, s), 6.74 (1H, dd, $J=3.0$ Hz and $J=8.6$ Hz), 6.92 (1H, d, $J=3.0$ Hz), 7.21 (1H, s), one non-visible proton in these experimental conditions. ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 30.4, 117.2, 117.9, 125.6, 130.9, 136.3, 154.4. LC/MS (ESI⁺): $t_r=2.69$ min, m/z : 219.2/221.6/223.49 [$M+H$]⁺. HRMS m/z [$M-H$]⁻ calcd for C_7H_6BrClO : 218.92178 Da, found: 218.92194 Da.

1.2.13. *Tert*-butyl[2-(2-{2-[(2-chloro-5-hydroxybenzyl)amino]ethoxy}-ethoxy)-ethyl]carbamate (**16**)

To a solution of NEt_3 (1 equiv., 1.5 mmol) and *tert*-butyl{2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate (1.3 equiv., 0.68 mmol) in THF (15 mL), a solution of **15** (1 equiv., 1 mmol) in THF (3 mL) was added portion wise. The reaction mixture was stirred at rt for 12 h. Water was then added (30 mL) and the mixture was extracted three times with CH_2Cl_2 (3 x 20 mL). The organic layer was washed six times with water (6 x 50 mL), dried over Na_2SO_4 , filtered, and evaporated. The crude residue was purified by silica gel column chromatography ($CH_2Cl_2/MeOH/NH_3$ 90/9.9/0.1) to afford an orange oil, yield: 76%, MW: 388.89 g/mol. 1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 1.42 (9H, s), 2.88-2.92 (2H, m), 3.24-3.31 (2H, m), 3.45-3.72 (8H, m), 4.94 (2H, s), 5.29 (1H, s), 6.63-6.69 (1H, m), 6.94 (1H, s), 7.13 (1H, d, $J=8.6$ Hz), two non-visible protons in these experimental conditions. ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 28.4 (3 CH_3), 40.4, 48.3, 50.5, 69.4, 70.2 (2 CH_2), 70.3, 79.5, 116.4, 117.2, 123.6, 130.2, 136.5, 156.0, 156.3. LC/MS (ESI⁺): $t_r=2.73$ min, m/z : 389.12/391.22 [$M+H$]⁺. HRMS m/z [$M+Na$]⁺ calcd for $C_{18}H_{29}ClN_2O_5$: 411.16572 Da, found: 411.16586 Da.

1.2.14. *Tert*-butyl{2-[2-(2-{[2-chloro-5-(2-trichloromethylquinazolin-4-yloxy)-phenyl]methylamino}ethoxy)ethoxy]ethyl}carbamate (**17**)

In a sealed vial, a solution of **16** (1 equiv. 0.51 mmol) in DMF (5 mL) was placed under nitrogen atmosphere. A solution of NaH (1.3 equiv. 0.67 mmol) in DMF (2 mL) was added and the reaction mixture was stirred at rt for 30 min. Next, a solution of 4-chloro-2-trichloromethylquinazoline (1 eq, 0.51 mmol) in DMF (5 mL) was added drop by drop. After 9 h at rt, water was added (100 mL) and the reaction mixture was extracted three times with AcOEt (3 x 20 mL). The organic layer was washed with water (50 mL), dried over Na_2SO_4 , filtered, and evaporated. The crude residue was purified by silica gel column chromatography (Petroleum ether/ $CH_2Cl_2/MeOH/NH_3$ 30/63/6/1) to afford a yellow oil, yield: 18%, MW: 634.38 g/mol. 1H NMR ($CDCl_3$, 200

MHz): δ (ppm) 1.40 (9H, s), 2.89-2.93 (2H, m), 3.25-3.30 (2H, m), 3.47-3.69 (8H, m), 4.04 (2H, s), 5.15 (2H, s), 7.28-7.32 (1H, m), 7.45 (1H, d, $J=8.7$ Hz), 7.59 (1H, s), 7.72-7.80 (1H, m), 7.95-8.03 (1H, m), 8.13 (1H, d, $J=8.4$ Hz), 8.36-8.40 (1H, m). ^{13}C NMR (CDCl_3 , 50 MHz): δ (ppm) 28.4 (3 CH_3), 40.3, 48.0, 50.3, 69.6, 70.1, 70.2, 70.3, 79.1, 96.6, 115.1, 121.8, 123.4, 123.6, 128.9, 129.2, 130.2, 130.5, 135.0, 137.4, 150.8, 151.0, 156.0, 159.8, 166.9. LC/MS (ESI⁺): $t_r=5.14$ min, m/z : 632.82/634.83/635.42/636.78 [M+H]⁺. HRMS m/z [M+Na]⁺ calcd for $\text{C}_{27}\text{H}_{32}\text{Cl}_4\text{N}_4\text{O}_5$: 655.10190 Da, found: 655.10139 Da.

1.2.15. 7-[(2-{2-[(2-aminoethoxy)ethoxy]ethyl}amino)methyl]-*N*-(2,4-dichlorophenyl)-2-trichloromethylquinazolin-4-amine (**18**)

A mixture of **11** (1 equiv., 0.88 mmol) and HCl in MeOH 2.4 M (5 mL) is stirred at rt during 2 h. The volatile was evaporated, and the crude residue was washed with ethylic ether (5 x 5 mL) to afford a beige solid, yield: 100%, MW: 567.75 g/mol. ^1H NMR ($\text{DMSO-}d_6$, 200 MHz): δ (ppm) 2.93-2.97 (2H, m), 3.11-3.12 (2H, m), 3.59-3.67 (4H, m), 3.79 (2H, t, $J=4.9$ Hz), 4.43 (2H, s), 7.51-7.55 (1H, dd, $J=2.4$ Hz and $J=8.5$ Hz), 7.68-7.72 (1H, m), 7.77-7.78 (1H, m), 8.02 (1H, dd, $J=0.8$ Hz and $J=7.9$ Hz), 8.14-8.17 (4H, m), 8.73 (1H, d, $J=8.5$ Hz), 9.85 (2H, ls), 10.72 (1H, ls). ^{13}C NMR ($\text{DMSO-}d_6$, 50 MHz): δ (ppm) 38.4, 45.7, 49.3, 65.6, 66.6, 69.5, 69.6, 97.5, 113.4, 124.9, 127.7, 129.1, 126.6, 129.9, 130.2, 131.3, 131.7, 134.4, 138.4, 148.9, 159.6, 160.5. HRMS m/z [M+H]⁺ calcd for $\text{C}_{22}\text{H}_{24}\text{Cl}_5\text{N}_5\text{O}_2$: 566.03726 Da, found: 566.03745 Da.

1.3. Synthesis of Lantern Matrices

1.3.1. General

HydroxyMethylPhenoxy (HMP) Lanterns PADHMP008[®] loading 8 μmoles were purchased from Mimotopes.

At the end of each step, in order to assess the quality of the anchoring, 2 lanterns were cleaved using a mixture of TFA/ CH_2Cl_2 (1/1) (0.5 mL). After 1 h at rt, the cleavage solution was then evaporated under vacuum, washed three times with CH_2Cl_2 and analyzed by NMR.

1.3.2. LM1

As previously described, [23] to 10 HMP lanterns in CH_2Cl_2 (5 mL) were added chloroacetyl chloride (80 μL , 0.2 M) and pyridine (80 μL , 0.2 M). The mixture was stirred at rt for 2 h. The lanterns were then successively washed with CH_2Cl_2 (3 x 5 mL), THF (3 x 5 mL), THF/ H_2O 1/1 (3 x 5 mL), THF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and ethylic ether (3 x 5 mL) to afford **LM1**.

1.3.3. LM2

To a pool of 8 **LM1**, was added a solution of 2,2'-[ethane-1,2-diylbis(oxy)]diethylamine (117 μL , 0.4 M) in DMSO (5 mL) for 2 h at rt. Lanterns were then successively washed with CH_2Cl_2 (3 x 5 mL), THF (3 x 5 mL), THF/ H_2O 1/1 (3 x 5 mL), THF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and ethylic ether (3 x 5 mL) to afford **LM2**.

1.3.4. LM3

To a pool of 6 **LM2**, was added a solution of 7-Bromomethyl-*N*-(2,4-dichlorophenyl)-2-trichloromethylquinazoline **10** (114 mg, 0.2 M) in DMF (5 mL). The mixture was stirred at rt for 12h. The lanterns were then successively washed with CH_2Cl_2 (3 x 5 mL), THF (3 x 5 mL), THF/ H_2O 1/1 (3 x 5 mL), THF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and ethylic ether (3 x 5 mL) to afford **LM3**.

1.4. Synthesis of beads matrices

Commercial CNBr Activated Sepharose® 4B (Sigma Aldrich) were used as beads supports.

1.4.1. Preparation of CNBr Activated Sepharose® beads

CNBr Activated Sepharose® beads (1g) were washed with a solution of HCl 1 mM (200 mL) for 15 min at rt. The beads were then placed in a solution of DMF/H₂O adjusted to pH = 8 using a NaHCO₃ saturated solution. The mixture was stirred at rt for 12h to afford **gel 1**.

1.4.2. Preparation of bead matrices (BM) 1 and 2

Gel 1 was washed with a solution of DMF/H₂O 1/1 (50 mL). Then, a solution of **18** in DMF/H₂O 1/1 (adjusted to final calculated concentrations of 3 µmol of **18**/mL resin and 6 µmol of **18**/mL resin) (50 mL) was added to **Gel 1** at pH = 8 and stirred 2 h at rt, to afford **BM1** (3 µM) and **BM2** (6 µM). **BM1** and **BM2** were then successively washed with DMF/H₂O (1/1, pH= 8, 50 mL), acetic acid (0.1 M, pH= 4, 50 mL), and DMF/H₂O (1/1, pH= 8, 50 mL). After coupling process, the remaining active groups of **BM1** and **BM2** were blocked in pH=8 using a solution of ethanolamine in DMF/H₂O (1M) under agitation for 2 h at rt. This operation was repeated once. The beads were then washed successively with acetic acid (0.1 M, pH= 4, 50 mL), DMF/H₂O (1/1, pH= 8, 50 mL), and three times with PBS. **BM1** and **BM2** were then stored in PBS supplemented with 0.05% NaN₃ to avoid microbial growth at 4°C until use.

2. Preparation of *P. falciparum* extracts

The *P. falciparum* clone 3D7 was cultured *in vitro* by standard methods. [46] Briefly, parasites were grown in human erythrocytes at 5% haematocrit in complete RPMI 1640 medium in 25-cm² ventilated flasks incubated at 37°C under 5% CO₂, using 0.5% Albumax II (Invitrogen) instead of human serum. [47] Asexual blood stage parasites were maintained at 0.5-6% parasitemia, parasitemia was measured daily by examining Giemsa-stained blood smears.

Cell-free parasite extracts were prepared from frozen saponin (15% v/v solution in PBS) -lysed *P. falciparum* pellets sonicated in M-PER Mammalian Protein Extraction Reagent (Pierce) supplemented with 1 mM phenylmethylsulphonyl fluoride and Complex™ mixture protease inhibitor tablet from Roche Applied Science. Lysates were cleared by centrifugation at 10,000 x g for 15 min at 4°C, and the total amount of protein in the supernatant was measured by the Bio-Rad protein assay.

3. Target fishing experiments [41,20]

3.1. Buffers

Homogenization buffer: The homogenization buffer was 60 mM β-glycerophosphate, 15 mM p-nitrophenyl phosphate, 25 mM Mops (pH 7.2), 15 mM EGTA, 15 mM MgCl₂, 1 mM dithiothreitol, 1 mM sodium vanadate, 1mM NaF, 1mM phenylphosphate, 0.1 % NP-40, 10 µg/ml of leupeptin, aprotinin and soybean trypsin inhibitor and 100 µM benzamidine.

Bead buffer: Bead buffer: 50 mM Tris pH 7.4, 5 mM NaF, 250 mM NaCl, 5 mM EDTA, 5 mM EGTA, 0.1 % NP-40, 10 µg/ml of leupeptin, aprotinin and soybean trypsin inhibitor and 100 µM benzamidine.

3.2. Preparation of Extracts

Cell-free parasite extracts were directly homogenized and processed for affinity chromatography or stored at -80 °C prior to use. *P. falciparum* parasites were homogenized and sonicated in homogenization buffer (2 ml/ number of parasites of material). Homogenates were centrifuged for 10 min at 14,000 g at 4 °C. The supernatant was recovered, assayed for protein content (Bio-Rad protein assay), and immediately loaded batchwise on the affinity matrix.

3.3. Affinity Chromatography

Just before use, 10 µL packed **BM (BM1 or BM2)** inhibitor (or 50 µl in 1/5) beads are washed with 1 mL bead buffer. The supernatant was removed and the *P. falciparum* lysate (volume depending on the concentration, adjusted to have 3 mg of proteins) was added. The volume was then adjusted to 1000 µL by adding bead buffer, and the tubes were rotated at 4°C for 30 min. After a brief spin at 10 000 × g and removal of the supernatant, the beads were washed four times with bead buffer before addition of 40 µL 2× Laemmli sample buffer. Following heat denaturation at 95°C for 3 min, the bound proteins were analysed by Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed by silver or Coomassie blue staining. The nature of the putative drug targets was analysed by mass spectrometry (MS). For this purpose, the protein bands are excised from the gel and further stored in a solution of acetic acid 0.1% (v/v) before their identification by MS.

The same protocol was applied with Lantern matrices, without centrifugation steps. Instead of 10 µl of BM, a module of HMP-Lanterns was excised and used.

4. Mass Spectrometry Analysis

The cysteine residues were reduced by 100 µL of 10 mM DTT at 56°C and alkylated by 150 µl of 55 mM iodoacetamide at room temperature. Iodoacetamide solution was replaced by 100 µl of 100 mM NH₄HCO₃ and gel dehydration was achieved with acetonitrile. After evaporation in Speed Vac (Thermo), proteins were digested overnight at 37°C by a solution containing 0.9 µg of a modified porcine trypsin (Promega, Madison, WI, USA) prepared in 25 mM NH₄HCO₃. Finally, a double extraction was performed, first with 5% (v/v) formic acid solution, and subsequently with 100% (v/v) ACN.

The resulting peptide mixture was dried under vacuum and resuspended by 50 µl of 1% formic acid solution.

The peptides mixtures were analysed by online nanoflow liquid chromatography tandem mass spectrometry (LC-MS/MS) on an EASY-nLC IITM system (Proxeon, Odense, Denmark) connected to the LTQ Orbitrap Discovery instrument (Thermo Fisher Scientific, Bremen, Germany). 10 µl of the peptide mixtures were concentrated onto the 2-cm pre-analytical column (300-µm inner diameter) packed with 5-µm C18 beads (C18PepMap100, Dionex). They were separated in a 15-cm analytical column (75-µm inner diameter) packed with 3-µm C18 beads (AcclaimPepMap100, Dionex) with a 60-min gradient from 5% to 35% acetonitrile in 0.1% formic acid. The effluent from the HPLC column was directly electrosprayed into the mass spectrometer. The LTQ Orbitrap instrument was operated in data-dependent mode to automatically switch between full scan MS and MS/MS acquisition. Instrument control was through Tune 2.5.5 and Xcalibur 2.1.

For the low-resolution CID-MS/MS top5 method, full scan MS spectra (from m/z 400–2000) were acquired in the Orbitrap analyzer after accumulation to a target value of 5e6 in the linear ion trap with resolution $r = 30,000$. The 5 most intense peptide ions with charge states ≥ 2 were sequentially isolated to a target value of 30,000 and fragmented in the linear ion trap by CID with normalized collision energy of 35% and wideband-activation enabled. Ion selection threshold was 500 counts for MS/MS, and the maximum allowed ion accumulation times were 500 ms for full scans in the orbitrap and 200 ms for CID-MS/MS measurements in the LTQ. An activation $q = 0.25$ and activation time of 30 ms were used.

Standard mass spectrometric conditions for all experiments were: spray voltage, 1.7 kV; no sheath and auxiliary gas flow; heated capillary temperature, 200 °C; predictive automatic gain control (AGC) enabled.

Sequences were deduced from the resulting fragment ion spectra using the PEAKS soft (Peaks Studio 5.3, Bioinformatics Solutions Inc.). The resulting peptide sequences were submitted to Peaks Search option to perform

protein identification into internal data banks or submitted to the BLAST program provided at the NCBI site to identify them by homology with proteins present in the databases.

5. *In vitro* antiplasmodial evaluation

A K1 culture-adapted *P. falciparum* strain resistant to chloroquine, pyrimethamine, and proguanil was used in an *in vitro* culture. It was maintained in a continuous culture, as described previously by Trager and Jensen. [46] Cultures were maintained in fresh A+ human erythrocytes at 2.5% hematocrit in a complete medium (RPMI 1640 with 25 mM HEPES, 25 mM NaHCO₃, and 10% of A+ human serum) at 37 °C under a reduced O₂ atmosphere (gas mixture: 10% O₂, 5% CO₂, and 85% N₂). Parasitemia was maintained daily between 1 and 3%. The *P. falciparum* drug susceptibility test was carried out by comparing quantities of DNA in treated and control cultures of parasites in human erythrocytes, according to a SYBR Green I fluorescence-based method [48] using a 96-well fluorescence plate reader. Compounds, previously dissolved in DMSO (final concentration less than 0.5% v/v), were incubated in a total assay volume of 200 µL (RPMI, 2% hematocrit and 0.4% parasitemia) for 72 h in a humidified atmosphere (10% O₂ and 5% CO₂) at 37 °C, in 96-well flat bottom plates. Duplicate assays were performed for each sample. After incubation, plates were frozen at 20 °C for 24 h. Then, the frozen plates were thawed for 1 h at 37 °C. A total of 15 µL of each sample was transferred to 96-well flat-bottom non-sterile black plates (Greiner Bio-One, Kremsmünster, Austria) already containing 15 µL of the SYBR Green I lysis buffer (2X SYBR Green I, 20 mM Tris base pH 7.5, 20 mM EDTA, 0.008% w/v saponin, 0.08% w/v Triton X-100). Negative control treated by solvents (DMSO or H₂O) and positive controls (chloroquine, doxycycline and artesunate) were added to each set of experiments. Plates were incubated for 15 min at 37 °C and then read on a TECAN Infinite F-200 spectrophotometer with excitation and emission wavelengths at 485 and 535 nm, respectively. The concentrations of compounds required to induce a 50% decrease in parasite growth (EC₅₀ K1) were calculated from three independent experiments.

6. Cytotoxicity assay

The HepG2 cell line was maintained at 37 °C, 5% CO₂, at 90% humidity in MEM supplemented with 10% fetal bovine serum, 1% L-glutamine (200 mM), and penicillin (100 U/mL)/streptomycin (100 µg/mL) (complete RPMI medium). The cytotoxicity of the tested molecules on the HepG2 (hepatocarcinoma cell line purchased from ATCC, ref HB- 8065) cell line was assessed according to the method of Mosmann [49] with slight modifications. Briefly, 5.10³ cells in 100 µL of complete medium were inoculated into each well of 96-well plates and incubated at 37 °C in humidified 5% CO₂. After 24 h incubation, 100 µL of medium with various product concentrations dissolved in DMSO (final concentration less than 0.5% v/v) were added and the plates were incubated for 72 h at 37 °C. Triplicate assays were performed for each sample. Each plate was then microscopically examined for possible precipitate formation before the medium was aspirated from the wells. Next, 100 µL of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) solution (0.5 mg/mL in medium without FBS) was added to each well. Cells were incubated for 2 h at 37 °C. After this time, the MTT solution was removed and DMSO (100 µL) was added to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (700 rpm) for 10 min. The absorbance was measured at 570 nm with 630 nm as reference wavelength using a BIO-TEK ELx808 Absorbance Microplate Reader (LabX, Midland, ON, Canada). DMSO was used as blank and doxorubicin (purchased from Sigma Aldrich) as positive control. Cell viability was calculated as a percentage of control (cells incubated without compound). The 50% cytotoxic concentration (CC₅₀) was determined from the dose–response curve, using TableCurve software 2D v.5.0. CC₅₀ values represent the mean value calculated from three independent experiments.

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Bibliography

- [1] World Health Organization, World Malaria Report **2022** online: <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022>.
- [2] Tilley, L.; Straimer, J.; Gnädig, N. F.; Ralph, S. A.; Fidock, D. A. *Trends Parasitol.* **2016**, *32*, 682–696.
- [3] Imwong, M.; Suwannasin, K.; Kunasol, C.; Sutawong, K.; Mayxay, M.; Rekol, H.; Smithuis, F. M.; Hlaing, T. M.; Tun, K. M.; van der Pluijm, R. W.; Tripura, R.; Miotto, O.; Menard, D.; Dhorda, M.; Day, N. P. J.; White, N. J.; Dondorp, A. M. *Lancet Infect. Dis.* **2017**, *17*, 491–97.
- [4] Balikagala, B.; Fukuda, N.; Ikeda, M.; Katuru, O. T.; Tashibana, S.-I.; Yamauchi, M.; Opio, W.; Emoto, S.; Anywar, D. A.; Kimura, E.; Palacpac, N. M. Q.; Odongo-Aginya, E. I.; Ogwang, M.; Horii, T.; Mita, T. *N. Engl. J. Med.* **2021**, *385*, 1163–1171.
- [5] Yang, T.; Otilie, S.; Istvan, E. S.; Godinez-Macias, K. P.; Lukens, A. K.; Baragaña, B.; Campo, B.; Walpole, C.; Niles, J. C.; Chibale, K.; Dechering, K. J.; Llinás, M.; Lee, M. C. S.; Kato, N.; Wyllie, S.; McNamara, C. W.; Gamo, F. J.; Burrows, J.; Fidock, D. A.; Goldberg, D. E.; Gilbert, I. H.; Wirth, D. F.; Winzeler, E. A.; Malaria Drug Accelerator Consortium. *Trends Parasitol.* **2021**, *37*, 493–507.
- [6] Medicine for Malaria Venture, online: <https://www.mmv.org/research-development/mmv-pipeline-antimalarial-drugs>.
- [7] Verhaeghe, P.; Rathelot, P.; Rault, S.; Vanelle, P. *Lett. Org. Chem.* **2006**, *3*, 891–897.
- [8] Verhaeghe, P.; Azas, N.; Hutter, S.; Castera-Ducros, C.; Laget, M.; Dumètre, A.; Gasquet, M.; Reboul, J. P.; Rault, S.; Rathelot, P.; Vanelle, P. *Bioorg. Med. Chem.* **2009**, *17*, 4313–4322.
- [9] Castera-Ducros, C.; Azas, N.; Verhaeghe, P.; Hutter, S.; Garrigue, P.; Dumètre, A.; Mbatchi, L.; Laget, M.; Remusat, V.; Sifredi, F.; Rault, S.; Rathelot, P.; Vanelle, P. *Eur. J. Med. Chem.* **2011**, *46*, 4184–4191.
- [10] Amrane, D.; Gellis, A.; Hutter, S.; Prieri, M.; Verhaeghe, P.; Azas, N.; Vanelle, P.; Primas, N. *Molecules* **2020**, *25*, 3929.
- [11] Desroches, J.; Kieffer, C.; Primas, N.; Hutter, S.; Gellis, A.; El-Kashef, H.; Rathelot, P.; Verhaeghe, P.; Azas, N.; Vanelle, P. *Eur. J. Med. Chem.* **2017**, *125*, 68–86.
- [12] Luth, M. R.; Gupta, P.; Otilie, S.; Winzeler, E. A. *ACS Infect. Dis.* **2018**, *4*, 301–314.
- [13] Carolino, K.; Winzeler, E. A. *Curr. Opin. Microbiol.* **2020**, *57*, 49–55.
- [14] Forte, B.; Otilie, S.; Plater, A.; Campo, B.; Dechering, K. J.; Gamo, F. J.; Goldberg, D. E.; Istvan, E. S.; Lee, M.; Lukens, A. K.; McNamara, C. W.; Niles, J. C.; Okombo, J.; Pasaje, C. F. A.; Siegel, M. G.; Wirth, D.; Wyllie, S.; Fidock, D. A.; Baragaña, B.; Winzeler, E. A.; Gilbert, I. H. *ACS Infect. Dis.* **2021**, *7*, 2764–2776.

- [15] Bopp, S.; Pasaje, C. F. A.; Summers, R. L.; Magistrado-Coxen, P.; Schindler, K. A.; Corpas-Lopez, V.; Yeo, T.; Mok, S.; Dey, S.; Smick, S.; Nasamu, A. S.; Demas, A. R.; Milne, R.; Wiedemar, N.; Corey, V.; De Gracia Gomez-Lorenzo, M.; Franco, V.; Early, A. M.; Lukens, A. K.; Milner, D.; Furtado, J.; Gamo, F. J.; Winzeler, E. A.; Volkman, S. K.; Duffey, M.; Laleu, B.; Fidock, D. A.; Wyllie, S.; Niles, J. C.; Wirth, D. F. *Nature Commun.* **2023**, *14*, 1455.
- [16] Schnier, J. B.; Kaur, G.; Kaiser, A.; Stinson, S. F.; Sausville, E. A. Gardner, J.; Nishi, K.; Bradbury, E. M.; Senderowicz, A. M. *FEBS Lett.* **1999**, *454*, 100–104.
- [17] Iwaoka, Y.; Nishino, K.; Ishikawa, T.; Hideyuki, I.; Sawa, Y.; tai, A. *Analyst* **2018**, *143*, 874–882.
- [18] Ciayadi, R.; Kelso, G. F.; Potdar, M. K.; Harris, S. J.; Walton, K. L.; Harrison, C. A. Haern, M. T. W. *Bioorg. Med. Chem.* **2013**, *21*, 6496–6500.
- [19] Tribouillard, D.; Gug, F.; Galons, H.; Bach, S.; Saupe, S. J.; Blondel, M. *Prion* **2007**, *1*, 48–52.
- [20] Doerig, C.; Meijer, L.; Mottram, J. *Trends Parasitols.* **2002**, *18*, 366–371.
- [21] Knockaert, M.; Gray, N.; Damiens, N.; Chang, Y.T.; Grellier, P.; Grant, K.; Fergusson, D.; Mottram, J.; Soete, M.; Dubremetz, J.F.; Le Roch, K.; Doerig, C.; Schultz, P.G.; Meijer, L. *Chem. Biol.* **2000**, *7*, 411–422.
- [22] Alves, E.; Nakaya, H.; Guimarães, E.; Garcia, C. R. S. *Curr. Res. Microbiol. Sci.* **2023**, *4*, 100179.
- [23] Guiffant, D.; Tribouillard, D.; Gug, F.; Galons, H.; Meijer, L.; Blondel, M.; Bach, S. *Biotechnol. J.* **2007**, *2*, 68–75.
- [24] <http://www.mimotopes.com/knowledgeBaseDocument.asp?did=80&cid=26,35,59>. *online available 01/30/23*.
- [25] Renault, J.; Lebranchu, M.; Lecat, A.; Uriac, P. *Tetrahedron Lett.* **2001**, *42*, 6655–6658.
- [26] Cosquer, A.; Pichereau, V.; Le Mee, D.; Le Roch, M.; Renault, J.; Carbon, B.; Uriac, P.; Bernard, T. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 49–54.
- [27] Van Wye, J.; Ghori, N.; Webster, P.; Mitschler, R.R.; Elmendorf, H.G.; Haldar, K. *Mol. Biochem. Parasitol.* **1996**, *83*, 107–120.
- [28] Quevillon, E.; Spielmann, T.; Brahimi, K.; Chattopadhyay, D.; Yeramian, E.; Langsley, G. *Gene* **2003**, *306*, 13–25.
- [29] Struck, N.S.; Herrmann, S.; Schmuck-Barkmann, I.; de Souza Dias, S.; Haase, S.; Cabrera, A. L.; Treeck, M.; Bruns, C.; Langer, C.; Cowman, A.F.; Marti, M.; Spielmann, T.; Gilberger, T.W. *Mol. Microbiol.* **2008**, *67*, 1320–1330.
- [30] Chattopadhyay, D.; Langsley, G.; Carson, M.; Recacha, R.; DeLucas, L.; Smith, C. *Acta Crystallogr. D Biol. Crystallogr.* **2000**, *56*, 937–944.
- [31] Gnädig, N.F.; Stokes, B.H.; Edwards, R.L.; Kalantarov, G.F.; Heimsch, K.C.; Kuderjavy, M.; Crane, A.; Lee, M.C.S.; Straimer, J.; Becker, K.; Trakht, I.N.; Odom John, A. R.; Mok, S.; Fidock, D.A. *PLoS Pathog.* **2020**, *16*, e1008482.

- [32] Schormann, N.; Hayden, K.L.; Lee, P.; Banerjee, S.; Chattopadhyay, D. *Protein Sci. Publ. Protein Soc.* **2019**, *28*, 1771–1784.
- [33] Zhang, M.; Wang, C.; Otto, T. D.; Oberstaller, J.; Liao, X.; Adapa, S. R.; Udenze, K.; Bronner, I. F.; Casandra, D.; Mayho, M.; Brown, J.; Li, S.; Swanson, J.; Rayner, J. C.; Jiang, R. H. Y.; Adams, J. H. *Science*, **2018**, *360*, 6388.
- [34] Chan, M.; Tan, D.S.H.; Sim, T.S. *Travel Med. Infect. Dis.* **2007**, *5*, 125–131.
- [35] Zhong, W.; Li, K.; Cai, Q.; Guo, J.; Yuan, M.; Wong, Y.H.; Walkinshaw, M.D.; Fothergill-Gilmore, L.A.; Michels, P.A.M.; Dedon, P.C.; Lescar, J. *Biochem. Biophys. Res. Commun.* **2020**, *532*, 370–376.
- [36] Kabri, Y.; Gellis, A.; Vanelle, P. *Green Chem.* **2009**, *11*, 201–208.
- [37] Bogert, M. T.; Hand, W.F. *J. Am. Chem. Soc.* **1906**, *28*, 94–104.
- [38] Bavetsias, V.; Henderson, E. A.; McDonald, E.; *Tetrahedron* **2007**, *63*, 1537–1543.
- [39] Kieffer, C.; Verhaeghe, P.; Primas, N.; Castera-Ducros, C.; Gellis, A.; Rosas, R.; Rault, S.; Rathelot, P.; Vanelle, P. *Tetrahedron* **2013**, *69*, 2987–2995.
- [40] Kabri, Y.; Verhaeghe, P.; Gellis, A.; Vanelle, P. *Molecules* **2010**, *15*, 2949–2961.
- [41] Yi Jin, L.; Ahn, J.H.; Lee, M. *J. Am. Chem. Soc.* **2004**, *126*, 12208–12209.
- [42] Chakraborti, A.K.; Sharma, L.; Shivani, R.G. *Tetrahedron*, **2003**, *59*, 7661–7668.
- [43] Miller, A.D. Triazoles. **1977**, *Ger. Offen.* DE 2648826A1 19770505.
- [44] Tanaka, A.; Mukoyoshi, K.; Kunikawa, S.; Takasuna, Y.; Maeda, J.; Chida, N.; Nagashima, S. **2010**, *PCT Int. Appl.* WO 2010024430 A1 20100304.
- [45] Keck, J.; Pueschmann, S.; Krueger, G.; Noll, K.R. **1974**, *Ger. Offen.* DE 2302967A1 19731011.
- [46] Trager, W.; Jensen, J.B. *Science* **1976**, *193*, 673–675.
- [47] Walliker, D.; Quakyi, I.A.; Wellems, T.E.; McCutchan, T.F.; Szarfman, A.; London, W.T.; Corcoran, L. M.; Burkot, T. R.; Carter, R. *Science* **1987**, *236*, 1661–1666.
- [48] Guiguemde, W.A.; Shelat, A.A.; Bouck, D.; Duffy, S.; Crowther, G.J.; Davis, P.H.; Smithson, D.C.; Connelly, M.; Clark, J.; Zhu, F.; Jimenez-Díaz, M.B.; Martinez, M.S.; Wilson, E. B.; Tripathi, A.K.; Gut, J.; Sharlow, E.R.; Bathurst, I.; Mazouni, F.E.; Fowble, J. W.; Forquer, I.; McGinley, P.L.; Castro, S.; Angulo-Barturen, I.; Ferrer, S.; Rosenthal, P.J.; DeRisi, J.L.; Sullivan, D.J.; Lazo, J.S.; Roos, D.S.; Riscoe, M.K.; Phillips, M.A.; Rathod, P.K.; Van Voorhis, W.C.; Avery, V.M.; Guy, R.K. *Nature* **2010**, *465*, 311–315.
- [49] Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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